



THE ACADEMY OF APPLIED
TECHNICAL STUDIES
BELGRADE



INTERNATIONAL SCIENTIFIC
AND PROFESSIONAL CONFERENCE
POLITEHNIKA 2023

CONFERENCE PROCEEDINGS

Belgrade, 15th December 2023



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SUSTAINABLE DEVELOPMENT**

**OCCUPATIONAL HEALTH
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SMART MANAGEMENT SYSTEMS

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FOREWORD

The International Scientific and Professional Conference POLITEHNIKA 2023 represents the seventh edition of the POLITEHNIKA scientific and professional events, occurring biannually since its inaugural event in 2011. POLITEHNIKA 2023 upholds a distinguished tradition and commitment to integrating higher education and practical application across a diverse spectrum of disciplines represented by defined thematic scopes.

Organized with the patronage of the Ministry of Education of the Republic of Serbia, the Ministry of Environmental Protection of the Republic of Serbia, the Ministry of European Integration of the Republic of Serbia, the Directorate for Occupational Safety and Health, the Office for Dual Education and National Qualifications Framework, the Conference of Academies of Applied Studies in Serbia, the Chamber of Commerce of Serbia, the Chamber of Commerce of Belgrade, the Institute for Standardization of Serbia, the Association of Belgrade Architects, the City of Požarevac and the Tourist Organization of the City of Požarevac, POLITEHNIKA 2023 stands as a collaborative platform at the intersection of academia, governmental institutions and industry.

This year heralds a notable progression with its international status and the incorporation of 10 conference scopes. Expanding beyond the thematic domains featured in previous events, the Conference now encompasses Environment and Sustainable Development, Occupational Safety and Health and Fire Safety, Smart Management Systems, Graphic Engineering, Design, Traffic Engineering, Biotechnology and Healthcare, Mechanical Engineering, Ecotourism and Rural development, and Mechatronics. By engaging experts, emerging professionals, and practitioners from these domains, the conference unifies fields of study programs of the Academy of Applied Technical Studies Belgrade. The thematic scopes, coupled with the structure of the compiled papers in this Proceedings, exhibit a rich diversity and multidisciplinary approach, fundamentally contributing to a holistic examination and resolution of societal and scientific challenges.

Comprising over 220 peer-reviewed contributions, the Proceedings represent a substantial intellectual asset, aligning with the conference's overarching objective of fostering the exchange of knowledge, research findings, and professional experiences among experts from industry, research institutions, and higher education establishments.

The Proceedings of the International Scientific and Professional Conference POLITEHNIKA 2023 serve as a comprehensive snapshot of the current landscape within the thematic realms of the conference, offering both insights and directives for ongoing scientific and professional development. Moreover, they proffer concrete solutions to practical challenges grounded in contemporary trends and pertinent insights.

The Academy of Applied Technical Studies Belgrade extends its sincere appreciation to all conference supporters whose financial contributions played a pivotal role in its successful realization. Special acknowledgment is reserved for the authors of the papers, whose diligence and eagerness to present their work to a wider audience, alongside the reviewers and members of the International Scientific Committee, Program Committee and Organizational Committee, have collectively contributed to the triumph of the International Scientific and Professional Conference POLITEHNIKA 2023.

Belgrade, December 2023
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ENVIRONMENT AND SUSTAINABLE DEVELOPMENT

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Srećko Stopić, PhD, Bernd Friedrich, PhD, Process Metallurgy and Metal Recycling, RWTH Aachen University, Germany

Advances in understanding of a role of unit metallurgical operations for recycling

Svetlana Grujić, PhD, Faculty of Technology and Metallurgy, University of Belgrade
Emerging pollutants in the environment: contamination of the Danube river basin in Serbia

Marija Nikolić, PhD, Faculty of Technology and Metallurgy, University of Belgrade
Biodegradable polyesters – from ecology to medicine

DESIGN

INVITED PAPER

Jelena Ristić Trajković, PhD, Faculty of Architecture, University of Belgrade
Society, Ecology and Design Education: Transformative Learning for Future Sustainable and Healthy Environments

MECHANICAL ENGINEERING

INVITED PAPERS

Tamara Bajc, PhD, Faculty of Mechanical Engineering, University of Belgrade
Energy savings and CO₂ emission reduction potential through the existing building renovation

Marko S. Jarić, PhD, Innovation Centre of Faculty of Mechanical Engineering in Belgrade
Analysis of remediation of horizontal cylindrical tank for oil storage

ECOTURISAM AND RURAL DEVELOPMENT

INVITED LECTURES

Marko Perić, PhD, Faculty of Tourism and Hospitality Management, University of Rijeka, Croatia
Challenges of sustainable tourism: Example of Croatia

Snežana Štetić, PhD, Balkan Network of Tourism Experts, Igor Trišić, PhD, Faculty of Geography, University of Belgrade
Selective forms of tourism and sustainable development of rural tourist destinations

INVITED PAPERS

Radomir Stojanović, PhD, Western Serbia Academy of Applied Studies
Education as a pillar of sustainable agritourism in Serbia

Jelena Premović, PhD, Faculty of Economics, University of Priština & Faculty of Economics and Engineering, University Business Academy in Novi Sad
Cultural heritage as a generator of sustainable development of tourism in local communities in the countries of the Western Balkans

Vladimir Živanović, Nevena Majstorović, Zlatibor Tourism Organization, Zlatibor
Analysis of the real number of tourist overnights based on the estimation of water consumption in Zlatibor

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INVITED PAPER

Andrea Matta, PhD, Dept. of Mechanical Engineering, Politecnico di Milano, Italy Mohsen Jafari, PhD, Dept. of Industrial and Systems Engineering, Rutgers University, USA
Towards a theory of digital twins: fundamental definition

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POLYCYCLIC AROMATIC HYDROCARBONS IN MEDICINAL HERBS: ANALYTICAL METHOD DEVELOPMENT

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are among the most toxic compounds known to man. Several PAHs are proven to be carcinogenic, mutagenic and teratogenic. Due to their wide distribution in the environment and their toxicity, it is considered important to monitor the levels of these compounds in foodstuff. PAHs have been detected in many foods including plant-based such as vegetable oils, cereal grains, herbs, spices, teas and supplements. As the awareness for the healthy lifestyle has increased globally, the intake of medicinal herbs as tea and spices has also grown immensely. Therefore, even stronger emphasis must be made to monitor the toxic levels of PAHs in herbs. The legislations of the maximum concentrations of certain contaminants in food of the Republic of Serbia included PAHs values in dry herbs only by the end of 2019 (Official Gazette RS, No. 81/2019) in comparison to European Commission Regulation dating from 2011. Therefore, herein we focused on analytical method development for PAHs determination in medicinal herbs.

Keywords: PAHs, medicinal herbs, validation, processing, HPLC-FLD

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of two or more condensed aromatic rings, they are toxic and have carcinogenic properties, and they are widespread in the environment [1]. PAH compounds can be produced naturally or anthropogenically. Naturally, a certain amount of PAHs in our region reaches the biosphere as a result of forest fires and hydrothermal processes. In urban [2] and industrial locations [3], PAHs are almost entirely emitted by human activity and are the main by-products of incomplete combustion of organic matter, and therefore become the main source of atmospheric pollution. These compounds from the air reach the soil and water environment through sedimentation or precipitation, from where they further reach fruits, vegetables and herbs, and finally the human body. People are exposed to PAHs in different ways. For non-smokers, the main source of exposure is the consumption of food and drink, as well as supplements. The special danger of PAHs for human health is due to their ability to bioaccumulate in the human body [4].

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The natural presence of wax on plant surfaces can concentrate low molecular weight PAHs, mainly through surface adsorption. The concentration of PAHs is generally higher on the surface of the plant (outer leaves, bark) than in the interior [5]. However, the origin of PAHs in fruits, vegetables and herbs is mainly of anthropogenic origin. Pollution of raw products (agricultural products) occurs during cultivation, growth, but also during transportation due to the deposition of aromatic hydrocarbons present in engine exhaust gases as well as during storage [6].

In order to put on the market products that are healthy and safe for human health, the criteria of standards and maximum allowed concentrations prescribed by the provisions of national and EU regulations must be met. There are a large number of different PAHs, however, for the research and monitoring of their concentrations in environmental samples (water, air, soil, treated waste, sludge) the European Union and the American Environmental Protection Agency (Eng. Environmental Protection Agency, EPA) defines only 16 of them as priority (European Commission, 2005). PAHs known for their carcinogenic, teratogenic and mutagenic properties are: benzo[a]anthracene and chrysene, benzo[b]fluorane, benzo[j]fluorane, benzo[k]fluorane, benzo[a]pyrene, benzo[gy]perylene, coronene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene and ovalene. Although these 16 PAHs are highly relevant for environmental monitoring programs, they have not entered food legislation. Historically, benzo[a]pyrene has been used as the sole marker of toxicity; however, data have shown that foods, herbs, and food supplements contain toxic PAHs without the presence of benzo[a]pyrene. A re-evaluation by the European Food Safety Authority (EFSA) suggests that at least a subset of four PAHs, designated as PAH4, should be monitored in food. PAH4 compounds are benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b]fluoranthene. In the Republic of Serbia, the maximum allowed concentration of PAHs is regulated by the Rulebook on Maximum Concentrations of Certain Contaminants in Food, Official Gazette RS, No. 81/2019, which entered into force in November 2019 [7]. The maximum permitted concentration of benzo[a]pyrene in dried herbs is 10 µg/kg, while the maximum permitted concentration for the sum of PAH4 compounds is 50 µg/kg.

The development of analytical methods for PAHs should now focus on this subgroup. In terms of analytical methods, EU legislation on food contaminants follows a "criteria approach". This means that the legislator does not prescribe specific analysis procedures, but allows laboratories to use different analysis methods, if they can be objectively shown to meet the analytical requirements prescribed by the relevant legislation. The analysis of PAHs in herbs is a challenging endeavour, as there are isobaric PAHs that interfere with the quantification of the PAHs of interest. Seeds and raw plants can be contaminated with PAHs through artificial drying and heating during processing if precautions are not taken.

2. MATERIAL AND METHODS

Preliminary tests based on a literature review and already existing standardized methods for determining the content of polycyclic aromatic hydrocarbons for other matrices such as water (SRPS EN ISO 17993:2008, ISO 28540:2011, SRPS EN 16691:2016) were performed on three different chromatography methods including measurements on a gas chromatograph, a gas chromatograph connected to a mass spectrophotometer and a high-performance liquid chromatograph (HPLC) with a fluorescence detector. After preliminary tests, HPLC with a fluorescence detector (HPLC-FLD) was chosen as a satisfactory chromatographic method according to the modified ERA 8310 standard. Determination of polycyclic aromatic hydrocarbons (PAHs) in medicinal and herbal plants was done by liquid chromatography with a fluorescence detector (LC-FLD) according to the modified ERA 8310 standard method.

2.1 Concentration range of the method

The concentration measurement range of the method for determining the content of PAHs, using the HPLC-FLD technique, is 4.0-200 µg/L.

2.2 Principle of the method

2.2.1. Polycyclic aromatic hydrocarbons from dried herb samples were extracted with methylene chloride in an ultrasonic bath. The extract was purified and evaporated to near dryness in the absence of light and the solvent exchanged with acetonitrile. Analytes were separated by liquid chromatography [8] and identified and quantified on a fluorescent detector (HPLC-FLD).

2.2.2. The methylene chloride extract is purified from the presence of plant pigments and fatty acids on a silica gel column.

2.3 Preparation of sample for testing

2.3.1. A 2.00 g sample of dried herbal tea was soaked in 20 ml of water and left to hydrate well for 2 hours. Then, by liquid-liquid extraction, the aqueous solution is extracted with 2x5 ml of methylene chloride. The collected organic extracts were purified over silica gel and evaporated almost to dryness in the dark and the residue was dissolved in 2 ml of acetonitrile in an autosampler vial.

2.4 Interferences

The cleanliness of laboratory glassware, solvents, and reagents was checked. For this purpose, extraction vials were taken and carried out the entire analytical procedure. The obtained value was the value of the blank sample, blank test. The value obtained by analysing the blank sample was not included in the calculation, but it did not exceed 10% of the sample value.

2.5 Reagents

2.5.1 Reagents: Solvent for extraction, pesticide grade, or organic pollutant residues, methylene chloride, CH₂Cl₂, Solvent for modification, quality for pesticides, or for residues of organic pollutants; Acetonitrile; Mobile phase solvent, pesticide grade, or organic pollutant residues; Acetonitrile; Water (deionized); Water in which the presence of interfering peaks at the lower limit of detection of the analytes examined by this method was not found. Solvents used for sample preparation (toluene/methylene chloride/cyclohexane/acetonitrile) were of chromatographic grade. Solvent chromatograms showed no PAH interfering peaks.

The stock standard solutions were prepared in the appropriate solvent and stored in vials with Teflon-lined stoppers in the refrigerator. Intermediate mix standard solutions were prepared by diluting the stock standard solutions with acetonitrile. Calibration standard solutions were prepared by diluting the intermediate standard solution with acetonitrile - concentration range for PAH 1.0-200 µg/L.

2.5.2 Instruments

HPLC-FLD (AgilentTechnologies 1260 FC-AS, Quaternary pump – AgilentTechnologies 1260 Infinity, Autosampler – AgilentTechnologies 1260 ALS, Tremostat for the column - AgilentTechnologies 1290 thermostat.); Agilent Technologies ZORBAX Extend C18 Reverse Phase Separation Column, Rapid Resolution HD, 1.8 µm (particle size), 150 mm (length) x 2.1 mm (inner diameter); Ultrasonic bath; Fridge.

3. RESULTS AND DISCUSSION

3.1 Method validation

The method for determination of the content of PAHs in dried herbs using the HPLC-FLD technique was applied and validated on the instrument Agilent 1260 FLD Spectra with a fluorescence detection according to the modified standard ERA 8310. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method and the standard method were determined using blank samples that were treated in the same way as real samples.

Table 1. Limit of quantification

Analyte	LOD*	LOQ*	LOQ (methods)
Benzo(a)anthracene	0.8	0.9	1.25
Chrysene	1.0	1.2	1.25
Benzo(b)fluoranthene	0.5	0.8	1.25
Benzo(a)pyrene	0.6	0.8	1.25

* data obtained on the basis of OpenLabCDS, LOD= ≤ 1.0 $\mu\text{g}/\text{kg}$, LOQ= ≤ 4.0 $\mu\text{g}/\text{kg}$

Linearity was tested in a range of different concentrations for each analyte individually on a series of standard solutions prepared according to a standard procedure. Linearity is confirmed because the correlation coefficient (R^2) is greater than 0.999.

Table 2. Linearity in the range 1.25-200 $\mu\text{g}/\text{L}$

Linearity		
Analyte	$y = Ax + B$	R^2
Benzo(a)anthracene	$y = 177.92x + 2.3658$	0.9997
Chrysene	$y = 21.175x - 0.0144$	0.9993
Benzo(b)fluoranthene	$y = 233.87x + 1.9446$	0.9999
Benzo(a)pyrene	$y = 453.54x + 6.2165$	0.9997

Recovery - spiked samples of dried elderberry were prepared by added known concentrations of each analyte and their content was determined.

Table 3. Recovery SANTE/11312/2021 70-120%; repeatability SANTE/11312/2021 %RSD $\leq 20\%$

Analyte	Recovery 1.25 $\mu\text{g}/\text{L}$		Recovery 20.0 $\mu\text{g}/\text{L}$			Recovery 100.0 $\mu\text{g}/\text{L}$			
	%	stdev	%	%	stdev	%RSD	%	stdev	%RSD
Benzo(a)anthracene	83.7	0.59	82.2	82.2	0.74	0.94	79.6	0.64	0.85
Chrysene	79.8	0.17	77.7	77.7	0.32	0.43	74.5	0.47	0.66
Benzo(b)fluoranthene	89.6	0.23	88.5	88.5	0.34	0.34	88.0	0.53	0.53
Benzo(a)pyrene	80.3	0.35	80.7	80.7	1.49	1.51	74.4	1.22	1.24

3.2 Method application

The content of polycyclic aromatic hydrocarbons was determined for four forms: benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene and chrysene, as their sum PAH4 in accordance with the regulations of the Republic of Serbia on maximum concentrations of certain contaminants published in the Official Gazette RS, No. 81/20191, Article 2 of Annex 2, Section 6, in force since November 2019. The maximum permitted concentration of benzo[a]pyrene in dried medicinal herbs is 10 $\mu\text{g}/\text{kg}$, while the maximum permitted concentration for the sum of PAH4 compounds is 50 $\mu\text{g}/\text{kg}$. Samples were collected after drying. From 25 analysed samples four different samples of medicinal herbs contained a higher concentration compared to the reference limit values of the corresponding regulation.

4. CONCLUSION

Determination of the content of benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene in dried medicinal herbal using the technique of liquid chromatography with a fluorescence detector (HPLC-FLD) according to the modified standard ERA 8310, can be considered validated and put into routine operation.

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